*Eco. Env. & Cons. 26 (2) : 2020; pp. (594-598)*
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*ISSN 0971–765X*

# Biological control of mosquitoes larvae *Culex quiuquefasiatus* under laboratory conditions

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(Received 10 November, 2019; accepted 15 December, 2019)

**ABSTRACT**

This study was conducted to study predatory efficiency and functional response of females fish Gambusia affinis, when feeding on different densities of larvae stages of mosquitoes *Culex quiuquefasiatus*. Larval stages of mosquitoes were treated with predators results were observed after 24 houres, treatment were conducted at 25 ± 2 °C temperature and humidity of 65% ± 5 and 12 hours of illumination. Fish *Gambusia affinus*, showed with 5 larvae from the first stage an average daily consumption of 5.00±1.22 larvae/day while 20 larvae of the first stage had an average daily consumption of 16.4 ± 3.17 larva / day, while 5 larvae of the fourth stage the average daily consumption was 3.5± 1.82 larvae / day and 20 larvae from the fourth stage the average daily consumption was 14.5 ± 2.52 larva/day. The increased funtional respone With increased prey density makes us classified the fish *Gambusia affinis* predator as type II of Holling (1959) division. The results of the study of the time taken by the predator in the search larvae prey for of the first and fourth stages for mosquitoes *Culex quiuquefasiatus* larvae that with increasing prey density of the rate from 5 to 20 larvae / predator / day led to a gradual decrease in the time of search forprey.

***Key words :*** *Culex quiuquefasiatus, Biological control, Mosquitoes*

## Introduction

As environmental awareness grew, pesticide dangers became known, many pesticides lost their effectiveness and most insect pests became resistant, the search for ways to control rather than exterminate pests was the correct environmental thinking (Mulligan, *et al*., 1983). This can be achieved using several biological means (Osman *et al*., 2000, although predators have been widely used in biological control of mosquito larvae, but some such as Gumbosia affinis may destroy the local community (Al-Akel and Suliman, 2011), its effectiveness would be reduced if the larvae were able to detect its presence and response (Roberts, 2012) need to develop non-toxic and safe alternatives to humans and animals is one of its methods of life control and is intended to reduce the population group of the pest or harmful species to the extent that it does not cause great harm to humans or animals or activities, Other than insects, fishes, bacteria, fungus and others (Mathew, 2017). The preditores of the mosquitoes with their four stages - egg, larvae, pupae, and whole insect by arthropod and other fishes is an essential and important part to eliminate and reduce its numerical (Mahatma, 2016). The larvae and the pupae are the most exposure to predators, where they are attacked by larvae of fishes (Huang and Vanlan Dingham, 2017). *Culex* species are common among different mosquitoes, and if some larvae re-

main after predation, they become the most dangerous species in the transmission of diseases (Derk, 2017). The functional response to predators is known the relationship between prey density and predation rate population in predator and prey systems describes the rate at which the predator kills its prey with different densities, which can determine the predator's ability to regulate the prey populations. It is one of the important tools in determining the success or failure of control programs (Roberts, 2012). The aim of this paper is to study the efficiency of predators *Gambusiaaffinis* and funtional respone consumption of various larvae.

## Materials and Methods

Multiple ponds have been selected in different locations in Salah AL-ddin Governorate where mosquitoes are frequent in these ponds because they are rich in organic matter. After preliminary water survey, different samples of these ponds were taken to cover the area to be studied, by a long-drawn scoop. Larvae were placed in plastic containers perforated to allow air access and were transferred to the laboratory and emptied into glass basins and supplied with chlorine-free water and yeast to feed larvae (30 mg per basin) and covered with soft membrane. The study was conducted on labarotery conditions at temperatures 25 ± 2 C, relative humidity 65% ± 5 and a 12 hour/day illumination period to the adult stage where mosquitoes were transported to aluminum cages (150 x 100 x 80 cm), designed by the researcher. The adult stages are fed on a concentrated glucose solution 7% continuously and when egg is neede. Plastic vials are then placed inside the cage containing a quantity of water to lay eggs. The eggs are then transferred to metal trays of 30 x 30 cm non-rustic dimensions to breed the larvae after filling them with water and to add food made from bread crumbs and coverd with soft membrane to protect them and not to allow any external insects to lay eggs in water. When the larvae reach the fourth stage and before they cannot be moved to the white plastic containers covered with the top with a tightly closed hole and when the complete insects are pulled out by a special suction device.

**Predatores collected**

The predator fish *G. affinis* collected from permanent water ponds in Salah -Eddin Governorate. Predators were collected between March and July 2019. The predators were kept in the laboratory in glass basins. The dimensions of these glass basins were 80 x 60 x 50 cm. The predators were periodically fed to mosquito larvae until the date ofexpermination.

**Predatory effectivness**

To determine the predatory efficacy of predator on the incomplete stages of mosquitoes, two treatments were established, two of which included different larval stage of mosquitoes as well as the control. Each treatment included four replicates. Controlled treatment containing the same number of larvae was without predator. Each treatment is done 50 larvae from each stage of larvae with one of the predators after being starved for 24 hours in a plastic container 30 x 20 x 10 cm filled with 500 mL of water and 24 hours after counting the number of larvae .The number of larvae Consumed by the predator and each larvae lost are calculated.

**Functional response to prey density**

For the purpose of estimating the functional response, the predator density relationship was used with the predator of the fish, *G. affinis* were used with larvae density of (5, 10 ,15 , 20 ). The same tiend was done with first and fourth stage as well as a control coefficient. Each treatment included four replicates. The number of larvae killed can be calculated by subtracting the number of larvae before the experiment after 24 hours of exposure to predators, four replicates were used for the predators and the control treatment without predators. Time can be found in the search for prey according to the equation setby. Ha = a. H. T search

Where = Ha number of larvae consumed

a = number of replicates H=larval density in studied repiate T=time of prey.

**Predatory effectiveness**

In this study, Table 1 showed predator fish *G. affinis* the highest average consumption was 44.23 larvae / day from the first stage, while the fourth stage howed less consumption than the rest of the stages with an average consumption of 23.45 larvae/day.

The experimental laboratory efficacy of predators of fish *G. affinis*, showed an increase in the mean stage of the first larval stage and a gradual decline of this medium in the fourth stage larval. The number of larvae of mosquito larvae significantly decreased, with all the larvae growing from first to

**Table 2.** Mean average efficiency of predator *G. affinis* against *C. quiuquefasiatus* larvae under laboratory condition

| Predator | Larvae stage | Average predator ± standard deviation |
|---|---|---|
| *G. affinus* | First larval stage | 44.23 ± 6.43 a |
| | Fourth larval stage | 23.45 ±4.2 b |

fourth of stage. There was a significant difference in the number preyed by predator fish *G. affinis*.

The results showed that the mosquito larvae by predator *G.affinis* against *C quiuquefasiatur* which was inversely proportional to the larval age under laboratory conditions. The highest predation efficiency was compared stages to the first and fourth larval stages and significantly decreased the fourth larvae for predators. This was due to the higher nutritional value for the first stages compared to the fourth stages (Farghal, Ahmed Ibrahim, 1979). The study agrees with the study carried out by (Legner and Fisher, 1980) that the larvae of the fish T. zilii The density of *C. pipines* mosquitoes was reduced by almost 100% during the summer months, and predation reached a high sustainable level during the warmer months. *T. zillii* caused a reduction of more than 80% of the first and second larvae and 65% and IV. This study is consistent with a study (1 El-Shazly, 1993). Which demonstrated the efficiency of larval larvae of *Tilapia nilotica, Tilapia galilae, Tilapia zillii, Tilapia aurea* in biological control of *Culex pipines*. In a study (Vandrep, 1994; Slomy *et al.*, 2019; Jasman *et al*., 2019) in South Africa, which showed that larvae of different types of Barbus fish were superior to Gambusia fish in the consumption of larvae of the larval stages of mosquitoes are the preferred food of these fish. This study agrees with his study (Slomy *et al.,* 2019). In a study in the Gambia River to assess the predatory efficiency of two species of fish *Tilapia guineensis* and *Epiplatys spilargyreius* in the larvae of different larvae of mosquitoes Anopheles proved that the predatory ability differed between the two species where it proved that *T. guineensis* more capable of predation type *E. spilargyreius* and proved that Female Anopheles Do not lay eggs in the presence of these two types of fish. In a study (Jasman *et al*., 2019), the efficacy of *Tilapia nilotica* was evaluated alone or in combination to control the different immature stages of *Aedes aegypti* dengue under laboratory conditions. Mosquitoes were collected from trenches and reared under laboratory conditions, and fish were collected from local breeding and adapted prior to this study. This study was done in four replicates using four treatments containing the first repeater on five fish and larvae, while the second replicates contained five fish and larvae. 50 larvae were used for each larval stage in each treatment and were calculated after 1 hour, 2 hours, 8 hours, 16 hours and 24 hours. The larvae preferred first and second stage larvae to feed them from larvae of other stages because of their size. The larvae were 35.06%, 27.4%, 7.9%, 1% in larvae 1 to 4 larvae, respectively (Vasilis and Lucas, 2009). The local fish species *Rasbora daniconius, Puntius ticto, Puntius conchon*i had the predatory efficiency of *Culex quinquefasciatus*. The predation rate ranged from 267 to 865 and 277 to 888, 261 to 854 larva / day for predator respectively. Through study (Shabah *et al*., 2015) in Greece where fish species *Gambusia affinis* and *G. holbrooki* showed different efficiency in different larvae larvae of *C. tritaeniorhynchus*. The predatory efficiency of the first and second stage of *G. affinis* was greater compared to *G. holbrooki* as well as to the third, fourth, and virgin stages *G. affinis* showed the greatest efficiency compared to the species *G. holbrooki.*

**Funtional Respone**

Functional response of predator fish female *G.*

**Table 3.** Average consumption of predator female *G.affinus*, for different densities of mosquitoes with *C. quinquefasciatus* in 24 hours

| Number of larvae | Larvae stage | Mean number of predatory larvae ± standard devition | Predator search time for prey/hour |
|---|---|---|---|
| 1.72 | 5.00 ± 0.84 d | First | 5 |
| 1.41 | 9.85 ± 1.61 bc | First | 10 |
| 1.33 | 13.80 ± 2.58 ab | First | 15 |
| 0.9 | 16.31 ± 2.32 a | First | 20 |

*Means in the column and attachment with the same letter did not differ significantly (ANOVA test at a significant level of 5%)

*affinis*, for different densities of the mosquitoes *C.quinquefasciatus*

The functional response curve for the first and fourth phases of the *G. affinis* (Fig. 1) showed increased number of larvae consumed at a decreasing rate with increasing numerical density as the response curve decreased gradually until it was equal. The number of larvae consumed by the predator species increased with increasing larval density as between the Table 3. The analysis of the results was statistically significant. The predator *G.affinis*, with 5 larvae of first stage *C. quinquefasciatus* mosquitoes, had an average of 5.00 larvae / day, and with the predator density increasing to 10, 15 larvae per day by the predator at 9.85 ,13 .80, larvae / day respectively. When there are 20 larvae, the predation decreased to a maximum of 16.31 larva / day. The study of the time taken by the small chalk thickness of female *G. affinis.* The fourthstage of *C. quinquefasciatus* with increased prey density of 5 to 20 larvae/predator/larvae resulted in a gradual decrease in the time of prey from 1.72 to 9 hours The number of larvae consumed by predator will increase with the increased density of these larvae (Table 4). The predator, with 5 fourt-stage larvae of *C. quinquefasciatus* average of 34.3 larvae / day, and with the predator density increasing to 10, 15, larvae per day. The number of larvae consumed by the predator increased by 7.53, 12.00 larvae/day On the other hand, when there were 20 larvae, predation increased to a maximum of 14.56 larvae/day. The study of the time taken by the thickness of the small. The search for the fourth phase larvae of mosquitoe *C. quinquefasciatus* density of the rate of 5 to 20 larva/predator/led to a gradual decline in the time of the search for the prey from 1.32 to 0.64 hours. All differences between daily prednisone rates at 5%. The study showed a strong positive correlation between the prey density and the number of prey consumed by the predator. This indicates the functional response of predator *G. affinis* and the different prey densitie.

Note that the number of larvae of the second and fourth stage of mosquitoes *C. quinquefasciatus* consumed by fish *G. affinus*, are increased by increasing mosquito larval densities but with decreasing acceleration as mosquito larvae consumed at the first density are between 5-20 higher than in higher densities. The curve corresponds to the second type II of the functional response that predisposes predators to the different densities of prey, which are determined by the saturation of the predator and the processing time, which is an inversely dense type. The criteria that have been developed must be a short processing time relative to the total research time. A study (Amanda *et al*., 2017) showed that local fish *Adphinusdispar, Rasbora daniconius* showed a functional response of the Type II type when exposed to different densities of the larval larvae of *Culex quinquefasciatus* mosquitoes. This study is consistent with the laboratory study carried out by (Haq and Yadav, 2011). Which showed that the functional response to different densities of *Culex pipiens* and *Anopheles stephensi* mosquitoes to predatory fish *Peciliareticulata* is based on the Second instar and the fourth Fourth instar of *C. pipiens*. Stephensi showed a response of Type II, A study of (Kumar and Hwang, 2006) and showed that local fish *Cyprinus carpio* and *Carassius auratus* showed a functional response of type II Type II when subjected to different densities 15,30,40 larvae of the different larvae of the mosquito *Culex pipines*, and it was noted that the prey consumes the whole body of the prey in the low densities of prey in the second and fourth stages of the larvae of mosquitoes, while in high densities we see the consumption of all prey or part of the prey, leaving the legs, head, and part of the chest area. This explains the increase in the number of lar-

**Table 4.** Average consumption of predator female *G. affinus*, for different densities of mosquitoes with *C. quinquefasciatus* in 24 hours

| Number of larvae | Larvae stage | Mean number of predatory larvae ± standard devition | Predator search time for prey/ hour |
|---|---|---|---|
| 1.32 | 3.43 ± 0.93 d | fourth | 5 |
| 1.13 | 7.53 ±1.42 cd | fourth | 10 |
| 1.12 | 12.00±1.71 bc | fourth | 15 |
| 0.64 | 14.56± 1.45 ab | fourth | 20 |

*Means in the column and attachment with the same letter did not differ significantly (ANOVA test at a significant level of 5%)

vae consumed in whole or in part by the predator that appears to reach a certain level that cannot kill or consume any larger numbers. This explains the level of the slope at densities between 5 - 20 larvae /predator l day.

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